Small Size, Brooding, and Protandry in the Apodid Sea Cucumber *Leptosynapta clarki*

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Abstract. The apodid sea cucumber *Leptosynapta clarki* exhibits the three commonly associated traits of small adult size (max. length 113 mm), brooding (intraovarian = viviparity), and hermaphroditism (protandric). Juvenile *L. clarki* are released from the ovary at a length of 1–2 mm in the early spring (April–May) and are reproductively active as males in the reproductive season (November) following their birth. In their second year, some individuals continue to reproduce as males, but others undergo protandric sex change to reproduce as females. Analysis of the relationship between size and sex revealed a "critical" size for sex change at a weight of 200–400 mg with a 1:1 sex ratio above 500 mg total weight. Transitional gonads with previtellogenic oocytes and mature spermatozoa were observed, suggesting that sex change is initiated prior to reproducing in the current reproductive season. A test of the allometric hypothesis on the association between small size and brooding found no evidence for scaling constraints on brood size in *L. clarki*. These allometric constraints may be avoided because of potentially low fertilization success and brooding within a distensible structure. The sequential hermaphroditism in *L. clarki* may additionally be a method to reduce inbreeding in a species with limited dispersal.

Introduction

Two characters of the adult have been associated with brooding in marine invertebrates: small size and the tendency towards hermaphroditism. Several hypotheses have been proposed to explain the association of small adult size with brooding. The energetic hypothesis of Chia

(1974) suggested that the trend for lecithotrophy, brooding, or viviparity in small animals resulted from the lower energetic reserves for gamete production. Strathmann and Strathmann (1982) described three categories of hypotheses on the association of small adult size and brooding related to (1) dispersal, (2) adult longevity and recruitment, and (3) the allometry of structures associated with gamete production and brood care. These hypotheses are not mutually exclusive and no single one is entirely satisfactory (Strathmann and Strathmann, 1982). However, of these four hypotheses, the allometry hypothesis has received the most attention, primarily because it can be tested within a single species.

The allometry hypothesis predicts that brooding will not occur in large species, because of spatial limitations on brood size with increasing animal size (Heath, 1977; Strathmann and Strathmann, 1982). In numerical terms, fecundity is likely to increase with the cube of length, but the space for brooding may increase with a lower scaling constant, resulting in insufficient space for brooding the embryos. Although originally proposed for an external brooder (Strathmann and Strathmann, 1982), the allometry hypothesis has been tested by comparing the relationship between animal size, egg number, and number of brooded embryos in several marine invertebrates (Daly, 1972; Rutherford, 1973; Menge, 1974; Ockelmann and Muus, 1978; Rumrill, 1982; Strathmann *et al.*, 1984; Kabat, 1985; Gremare and Olive, 1986; McGrath and ÓFoighil, 1986; Russell and Huelsenbeck, 1989; Byrne, 1991; Brey and Hain, 1992; Hines, 1992; Hess, 1993).

Hermaphroditism, in which both male and female gametes are produced by a single individual in its lifetime, is common in marine invertebrates and is often associated with brooding (Ghiselin, 1969, 1974; but see Heller, 1993, for arguments against this association in gastropods). Ghiselin (1969, 1974) proposed three models for the evo-

Table I

Patterns of sex change and reproductive characters in the phylum Echinodermata

Species	Sex change	Brood[1]	Evidence for sex change[2]	Reference
Class Asteroidea				
Fromia ghardaqana	Protandric	N	H	Achituv and Delavault (1972)
Asterias forbesi	Protandric	N	L	Aldrich and Aldrich (1955)
Asterina gibbosa	Protandric	N	F², H	Bacci (1951)
	Protogynic	N	?	Bacci (1965)
	Protandric	N	H	Emson and Crump (1978)
Asterina pancerii	Protogynic	?	H	Cognetti (1954)
Patiriella exigua	Protandric	N	F¹, F²	Byrne (1992)
Class Holothuroidea				
Holothuria atra	Protandric	N	F¹, F²	Harriott (1982)
Labidoplax media	Protandric	?	F¹	Gotto and Gotto (1972)
Leptosynapta inhaerens	Protandric	N	F¹	Runnström (1927)
Leptosynapta clarki	Protandric	Y	H; F¹, F², H	Everingham (1961); Sewell (1991); herein
Class Ophiuroidea				
Ophiolepis kieri	Protandric	Y	F¹, F²	Hendler (1979)
Amphiura stepanovii	Protandric	Y		Fedotov (1926)
Ophiocantha bidentata	Protandric	N	F², H	Tyler and Gage (1982)
Ophionereis olivacea	Protandric	Y	F², H	Byrne (1991)
Ophiothrix sp.	Protandric	Y	F²	Schoppe and Holl (1994)

Species suspected of undergoing sex change: Brooder—*Pseudopsolus macquariensis* (Simpson, 1982); Nonbrooder—*Ophioplocus (Ophiceres) incipiens* (Mortensen, 1936); *Ophiothrix fragilis* (Gostan, 1956).

[1] Presence or absence of brooding based on review of Giese *et al.* (1991); Y = yes; N = no; ? = unknown.

[2] F = Field collections; F¹ = skewed sex ratio; F² = size/sex relationship; H = histology; L = laboratory observations.

lution of hermaphroditism. (1) The low-density model proposes that in sessile or slow-moving species, or in low-density populations, simultaneous hermaphroditism is advantageous because each contact of mature individuals can lead to reproduction (Tomlinson, 1966; Ghiselin, 1969). (2) The size-advantage model proposes that sequential hermaphroditism occurs when an individual can reproduce most efficiently as a member of one sex when small or young, but as a member of the other sex when it gets older and larger. Originally proposed by Ghiselin (1969), this has been extensively modeled (*e.g.,* Warner, 1975; Leigh *et al.,* 1976; Charnov, 1982, 1986; Iwasa, 1991). (3) The gene-dispersal model proposes that when motility is low, both the number and variety of potential mates are limited, and the genetic environment may be affected. Ghiselin (1969) discusses two particular effects: sequential hermaphroditism, which could reduce sibling crosses (Ghiselin, 1969), and simultaneous hermaphroditism, which could increase effective population size (Murray, 1964).

More recently, Heath (1977, 1979) predicted that if the space for brooding was limited and could hold fewer embryos than the individual could produce, then the remainder of reproductive resources could be reallocated towards male function as a simultaneous hermaphrodite. Data collected in *Asterina phylactica* were inconsistent with this hypothesis and led Strathmann *et al.* (1984) to propose that the correlation between brooding and simultaneous hermaphroditism was related to levels of inbreeding.

Although most brooding echinoderms are simultaneous hermaphrodites, recent research has suggested an association between brooding and sequential hermaphroditism within this phylum. In a comprehensive review of sex-changing species, Policansky (1982) cited evidence that 10 species of echinoderms change sex, the majority being protandric; two of these are brooders (Table I). Since that review, two protandric brooding ophiuroids (Byrne, 1991; Schoppe and Holl, 1994) and a protandric nonbrooding asteroid (Byrne, 1992) have been described (Table I). There are also reports of one brooding and two nonbrooding echinoderms that may change sex (Table I).

Leptosynapta clarki is a small (maximum length 113 mm), apodid sea cucumber found on the intertidal mudflats of Grappler Inlet, Bamfield, Vancouver Island, Canada. *L. clarki* has an annual reproductive cycle, brooding pentactulae in the ovary from November to April or May (Everingham, 1961; Sewell and Chia, 1994). Abundant sperm found in the ovaries of *L. clarki* sampled in False Bay, San Juan Island, Washington, in March or April (Everingham, 1961; Eckelbarger and Young, 1992) led Everingham to suggest that this species might be a protandric hermaphrodite. The present study confirms that *L. clarki* is protandric, describes the population con-

sequences of that sex change, and examines the hypotheses for the association between small adult size and brooding in marine invertebrates.

Materials and Methods

Collections and size measurements

Specimens of *Leptosynapta clarki* were collected at two sites in Grappler Inlet, Bamfield, British Columbia (Site 1: 48°49′57″ N. 125°06′45″ W; Site 2: 48°50′ N, 125°06′36″ W). Site 1 is located on the northern side of Barge Bay and has a short intertidal area (*ca.* 20 m) and a high proportion of gravel in the substrate. Site 2 is a gentler sloping mudflat (intertidal area *ca.* 60 m) at the opposite end of the channel separating "No-name" Island from Vancouver Island proper (see Sewell and Chia, 1994, Fig. 1). This site has a finer sediment composition with little gravel and contains *L. clarki* of a larger size than Site 1 (mean total weight ± SD: Site 1: 307.8 ± 153.99 mg, N = 720; Site 2: 490.4 ± 268.80 mg, N = 660; Sewell and Chia, 1994). Both sites have extensive *Zostera* beds in the lower intertidal that extend into the shallow subtidal.

The sea cucumbers were collected on the mid-intertidal mudflats, either by using cores or by sweeping away the upper 5 cm of sediment to reveal the animals in their burrows; put in buckets with the surrounding mud; and transported to the laboratory. Adults were removed from the sediment with a 2- or 0.85-mm sieve. The remaining fraction was then sieved through a 0.25-mm sieve to remove juveniles. Sea cucumbers were kept in seawater until length and weight were determined.

Animals to be measured were placed in a 90-mm petri dish containing 2.5% $MgCl_2$ in seawater (w/v). Length was measured, in millimeters, from the anterior end of the calcareous ring to the posterior. Sea cucumbers less than 20 mm were measured on a dissecting microscope equipped with an eyepiece graticule calibrated against a stage micrometer. Larger sea cucumbers were gently held straight with tweezers and length was measured to the nearest millimeter with reference to a small plastic ruler under the petri dish. After measurement of length, the sea cucumber was placed on a tissue to remove external water and then weighed on a fine-scale balance to determine its total weight (TW) in milligrams.

Size-sex relationship

The relationship between size and sex in *L. clarki* was determined in July 1991 when the gonads were well developed (Sewell and Chia, 1994). After a specimen was weighed, its sex was determined by examining the color of the gonads through the semitransparent body wall. In July, females had yellow ovaries with large eggs (*ca.* 200 μm) and males had white testes (Sewell and Chia,

1994). Juvenile sea cucumbers or those with small gonads were dissected to verify sex identification.

The relationship between sea cucumber size and sex was determined by calculating the percentage of females in each weight or length class. For weights less than 800 mg the data were divided into 50-mg size classes. Few sea cucumbers were found above 800 mg, so the size class was increased to 100 mg. The final category was sea cucumbers with weights > 1300 mg. Sea cucumbers in this category ranged from 1412 to 1684 mg (N = 4), so are plotted as a single point at 1500 mg. Sea cucumber length was divided into 5-mm size classes until 100 mm; the three sea cucumbers with greater length (104, 108, 113 mm) are plotted as a single point at 110 mm. The total number of sea cucumbers sampled was 693 (454 males, 239 females).

The breeding sex ratio was determined from haphazard cores used in following the recruitment of *L. clarki* on the intertidal mudflats at Site 1 (Sewell, 1993a). Ten haphazardly thrown cores were taken on the mid-intertidal in Barge Bay (Sewell, 1993a), the animals removed by sieving, and the size and sex of each sea cucumber determined in four samples during the winter of 1990–1991 and the summer of 1991.

Gonadal changes

Sea cucumbers sampled during regular gonad index dissections in January and February 1990 and 1991 (Sewell and Chia, 1994) were used to determine the size of animals with transitional gonads (that is, animals in the process of changing from male to female). Sea cucumbers were collected from Sites 1 and 2 (Total N = 240) and weighed and dissected as described in Sewell and Chia (1994). The gonad in *L. clarki* consists of two separate tubules that join through the dorsal mesentery to a single gonoduct (Sewell and Chia, 1994). Both gonad tubules were dissected, placed on a glass slide (38 × 75 mm) with a small amount of seawater, and temporarily mounted with a coverslip (35 × 50 × 0.02 mm). The weight of the coverslip revealed details of the gonad, such as the presence of oocytes, without the need for histology. Of the 240 sea cucumbers dissected, 87 were females brooding pentactulae or undergoing resorption. Of the remaining 153 sea cucumbers, 75 had transitional gonads; these were divided into 50-mg size classes for analysis.

Histological descriptions of protandric sex change were obtained during a detailed study of reproduction in *L. clarki* (Sewell and Chia, 1994). Gonads were preserved in Bouin's fixative, stored in 70% ethanol, and embedded in Paraplast. Sections were cut at 7 μm, stained in hematoxylin and eosin, and photographed on a Zeiss photomicroscope.

Allometry of reproduction

The relationship between the size of the female and the number of unfertilized eggs was determined in late October 1989 and 1990 prior to the spawning period in November and December (Everingham, 1961; McEuen, 1986; Sewell and Chia, 1994). Sea cucumbers were collected from Sites 1 and 2 and sieved from the sediment as described above. After total weight (TW) was measured, a longitudinal incision was made from the base of the calcareous ring to the posterior, the coelomic water was drained from the body cavity, and the animal was reweighed to obtain its drained weight (DW) in milligrams. The gonad was dissected and temporarily mounted under a coverslip. Counts were made of the total number of mature eggs per gonad ($N = 2$ tubules) in 20 females at two sites in each year (Total $N = 80$ females).

The relationship between the size of the female and the number of fertilized eggs/embryos was examined in sea cucumbers in late November and early December 1989 and mid-November 1990. This was during the spawning period of *L. clarki* at Bamfield (Sewell and Chia, 1994) but before degeneration of unfertilized eggs or significant pentactulae mortality (Sewell, 1993a). Counts were made of the number of unfertilized eggs, early stage embryos, or pentactulae in each gonad for 50 females. The allometric relationship between female size and number of pentactulae was determined for those females that had early embryos or pentactulae present ($N = 28$).

The allometric relationship between size and number of eggs or pentactulae was tested using a Model II reduced major axis (RMA) regression. Ordinary least squares (OLS) regressions were calculated using Microsoft Excel Version 3.0. Because of the considerable error in measuring size in sea cucumbers (Sewell, 1990), OLS regression will underestimate the value of the slope (see McArdle, 1988; LaBarbera, 1989), and may suggest that scaling constraints exist when there are none (Hess, 1993). RMA regressions were performed on raw and natural log ($\log_e$) transformed data. Standard error (s^2) and asymmetric confidence intervals were attached to the slope of the RMA regression using the formulas in McArdle (1988).

If there are no constraints on brood size, the number of embryos brooded is assumed to scale with body size in the same way as the fecundity of broadcast spawning animals; *i.e.*, linearly with body weight or the cube of length (Strathmann and Strathmann, 1982). To test whether brood size was limited by scaling constraints, the slope of the RMA line was thus compared to the slope predicted by isometry ($b = 1$ for the relationship between $\log_e$ body weight and $\log_e$ number of pentactulae) using a Student's t test (Clarke, 1980; McArdle, 1988). A slope less than 1 in this relationship would indicate scaling constraints on brood size in larger sea cucumbers.

Results

Size-sex relationship

Leptosynapta clarki is a protandric hermaphrodite in which sex change occurs over a broad range of size (Fig. 1). Sea cucumbers in the smallest weight class (0–50 mg) are juveniles of the year (Sewell, 1993a) and are all male (Fig. 1A). The smallest females found weighed 77, 120, and 150 mg (21, 36, 30 mm in length, respectively; Fig. 1).

Sex change did not occur in a distinct weight class (Fig. 1A). Few females were found until 200–250 mg total weight (Fig. 1A). Between 200 and 400 mg there was an increasing percentage of females in each size class. This percentage stabilized at about 50%, at weights above 500 mg (Fig. 1A).

A similar increase in percent females was seen with increased sea cucumber length (Fig. 1B). No females were found until the 20–25 mm size class. The percentage of females increased between 25 and 45 mm, plateauing at 50%, above a length of about 50 mm (Fig. 1B).
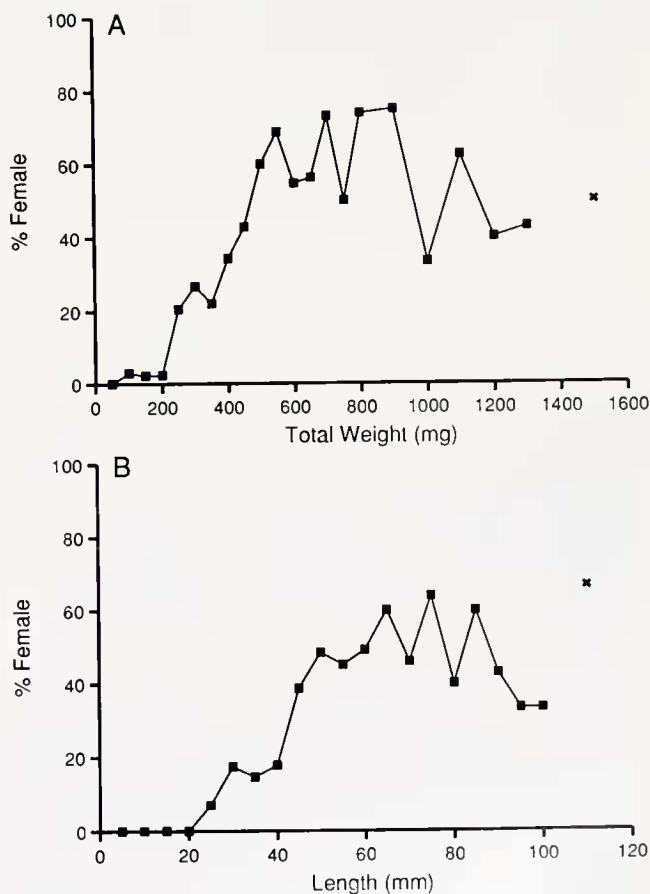

Figure 1. *Leptosynapta clarki.* Percent female sea cucumbers from Grappler Inlet, Bamfield, in size classes of (A) total weight (mg) and (B) length (mm). Point is plotted at upper limit of size class except for final category shown with (x); see details in text.

The presence of large numbers of small, but sexually active, males in the population strongly biases the sex ratio in *L. clarki* (Table II). In all samples the sex ratio was above 70% male, with a maximum of 88.5% male in August (Table II).

Gonadal changes

Most sea cucumbers with transitional gonads in January or February were in the 200–400 mg size range (Fig. 2). Sex change was, however, observed in some very large specimens (Fig. 2). The largest sea cucumber found with a transitional gonad weighed 855 mg (TW).

After spawning, the testes became thin and yellow, and shrank in length towards the gonad basis (Sewell and Chia, 1994). In protandric males, new oocytes were observed in fresh gonad tubules at the basal end adjacent to the gonad basis (Fig. 3A). Unspawned sperm were often present in the tubules with the new oocytes (Fig. 3B) and were active when exposed to seawater.

Histological examination of male gonads revealed that oocytes were present along the tubule wall in some mature testes (Fig. 3C). The testis lumen was packed with mature spermatozoa, whereas previtellogenic oocytes 10 to 20 μm in diameter were found along the tubule wall (Fig. 3C). After the spermatozoa were spawned, the oocytes continued to grow while the remnant spermatozoa were resorbed (Fig. 3D). Sex change must, therefore, be initiated prior to spawning in the current reproductive season.

Allometry of reproduction

Using raw and $\log_e$-transformed variables, reduced major axis (RMA) regressions were calculated between female size and number of eggs or pentactulae (Table III). In the period before spawning, female sea cucumbers show a positive significant relationship between weight (TW or DW) and egg number (Fig. 4, Table III). Regressions using natural log transformations of these variables are also significant, although there is a slight reduction of the r^2 in both cases (Table III). The allometric exponent of the $\log_e$-transformed egg number/drained weight relationship
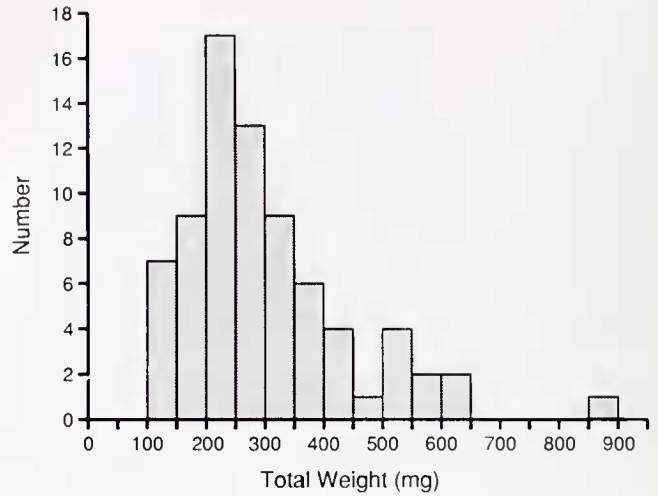

Figure 2. *Leptosynapta clarki.* Number of transitional gonads in each 50-mg total weight class during January and February 1990 and 1991. $N = 75$ sea cucumbers.

is 1.46, with 95% confidence intervals being 1.3–1.65 (Table III).

Lengths of the females used in the egg number/weight relationship were not recorded. These lengths were estimated using the OLS regression between total weight and length determined from the size/sex relationship described above (Length = 0.0595 (TW) + 20.519, $F = 2393.37$, df = 1,691, $p < 0.0001$, $r^2 = 0.776$). For this analysis, OLS regression is appropriate because the value for length is being estimated from the measurement of another variable (LaBarbera, 1989). A significant relationship was found between length and number of eggs in raw and natural log regressions, with an allometric exponent of 2.465 in the $\log_e$ transformation (Table III).

Weak relationships were found between female size and the number of pentactulae brooded (Table III). The only significant relationship was between drained weight and number of pentactulae in raw and $\log_e$-transformed data (Table III). Both regressions have low r^2 values (< 0.4, Table III). The allometric exponent of the $\log_e$-transformed regression was 1.84 (Table III). This slope is significantly greater than 1 ($T = 4.772$, df = 26, $p < 0.001$), indicating that fecundity is not proportionately smaller in larger sea cucumbers. In fact, larger females are producing disproportionately larger broods. This suggests that there are no scaling constraints on brood size in *L. clarki.*

Fertilization success in female *L. clarki* was variable. During the period sampled, 22 of the 50 females (44%) were unfertilized (0% fertilization). The remainder had percent fertilization ranging from 4.18–98.49% (mean ± SD = 47.74 ± 25.37%) with the mode at 40–50% fertilization (Fig. 5). There was no relationship between female weight and the percent fertilization (TW: $F = 0.193$, df = 1, 26, NS; DW: $F = 0.986$, df = 1, 26, NS).

Table II

Breeding sex ratio of Leptosynapta clarki *at Site 1, Grappler Inlet*

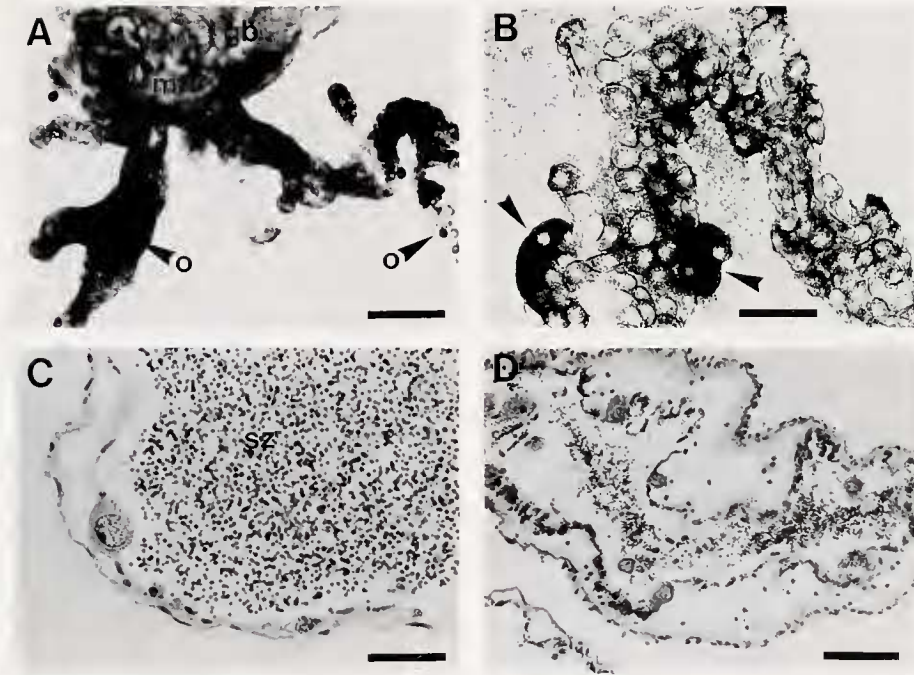| Date	% Male	Sex Ratio (Male:Female)	N
7 December 1990	70.5	2.4:1	112
7 February 1991	85.3	5.8:1	143
15 July 1991	85.9	6.1:1	128
12 August 1991	88.5	7.7:1	96
Mean	82.6		
Std. Deviation	8.2		

Figure 3. *Leptosynapta clarki.* Photomicrographs of transitional gonads. (A) Small resorbing testis in process of sex change. Almost the entire gonad is shown. Note the swollen gonad basis (gb) with madreporite (m), and small oocytes (o) in the tubules. Bar = 500 μm. (B) Transitional testis. Tubule contains numerous oocytes and remnant packets of sperm (arrow). Some sperm have broken from tubule onto base of slide. Bar = 200 μm. (C) Histological section of mature testis with spermatozoa (sz) in the lumen and early oocytes forming along the tubule wall. Section stained with hematoxylin/eosin. Bar = 25 μm. (D) Histological section of postspawned testis in transitional stage. Some remnant spermatozoa are seen in the lumen. Oocytes are present along the tubule wall. Bar = 50 μm.

Discussion

Protandry in Leptosynapta clarki

The histological and population information presented here confirms that *Leptosynapta clarki* is a protandric hermaphrodite and provides the first direct evidence of sex change within the class Holothuroidea. Two other holothurians in the order Apodida have been suggested to be protandric, though the evidence in both cases is somewhat circumstantial. *Leptosynapta inhaerens* was believed to be protandric because only 4 of 80 spawning specimens shed eggs (Runnström, 1927; cited in Hyman, 1955), and the lack of males in a sampled population of *Labidoplax media* led Gotto and Gotto (1972) to suggest that only very small and young specimens produce sperm.

There are few reports of sex change in the five remaining holothurian orders. Despite some anecdotal reports of protandry among dendrochirote holothurians (Smiley *et al.,* 1991), protandry has not been clearly demonstrated in any species. In aspidochirote holothurians, protandry is suggested in *Holothuria atra* on the basis of a change in the sex ratio with increased size (Harriott, 1982). How-

ever, sex change in *H. atra* is difficult to demonstrate because the species also reproduces asexually by binary fission and because the gonads regress after each spawning, making determination of the previous sex impossible (Harriott, 1982).

Sex allocation theory predicts that sex change should be an "all-or-nothing" response, with the animal or plant producing gametes of one sex until it is more profitable to produce gametes of the other sex, and then switching entirely to the latter (Charnov, 1982). Research on marine invertebrates has, however, shown a number of species that do not display an all-or-nothing response but have a 1:1 sex ratio above a certain size (*Patella argenvillei*—Branch, 1981; *Lottia gigantea*—Wright, 1989; *Patella kermadecensis*—Creese *et al.,* 1990; present study), or a wide size range or time over which sex change occurs (*Fromia ghardaqana*—Achituv and Delavault, 1972; *Crepidula fornicata*—Hoagland, 1978; *Ophionereis olivacea*—Byrne, 1991; *Coralliophila violacea*—Soong and Chen, 1991; *Patiriella exigua*—Byrne, 1992). The debate continues as to whether this is the result of a genetically programmed sex change, an environmental sex determination, or both.

One explanation applied in a number of marine invertebrates is that there are two sexual types within a species. Bacci (1951), who proposed this terminology in *Asterina gibbosa*, described one "race," or type, whose individuals (known as balanced hermaphrodites) change sex at a specific time in their existence. The other type contains two categories: unbalanced hermaphrodites, which change sex earlier or later, depending upon the individual, and true males and females, which remain one sex all their lives (Bacci, 1951). In the latter type, the presence of "true" males or females suggests a genetic predetermination for that sex. Evidence for predetermination is seen in *Bonellia viridis:* 10% of the larvae of this echiuran develop as males even if the "male determining factors" of a female proboscis are absent (Leutert, 1975). Similarly, in *Patella vulgata,* some individuals never change sex (Orton, 1928; Ballantine, 1961), and in *Lottia gigantea,* about 15% of the population change sex annually, regardless of environmental setting or age (Lindberg and Wright, 1985).

In *L. clarki,* all individuals start their reproductive lives as males (Sewell, 1991; Sewell and Chia, 1994; Sewell, 1993a); thus any "true sex" individuals (those that do not change sex during their lifetimes) in the population would be male. The rest of the population must be divided into those that change sex at some specific time, and those that change sex under the influence of some genetic or environmental factor. Male *L. clarki* may either delay sex change, as shown by the large size of some transitional males (Fig. 2), or have a labile sexuality that alternates between male and female. There is some circumstantial evidence that sex can be reversed from female to male (Sewell and Chia, 1994), and it is conceivable that sex may change more than once in the lifetime of a holothurian (Everingham, 1961; Harriott, 1982).
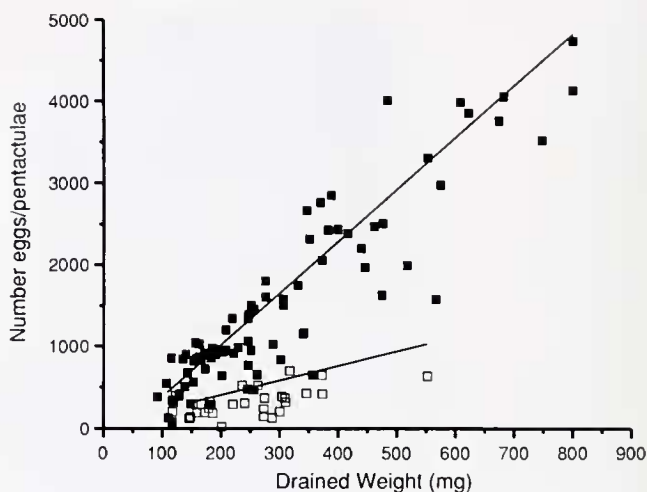

Figure 4. *Leptosynapta clarki.* Relationship between drained weight (mg) and number of eggs (filled squares) or pentactulae (open squares). RMA regressions:
Eggs: $y = 6.348x - 237.726$, $r^2 = 0.829$, $N = 80$.
Pentactulae: $y = 1.795x + 44.960$, $r^2 = 0.375$, $N = 28$.

The size-sex relationship presented here is a static data set that combines sea cucumbers of many ages within a size class (Fig. 1). Juveniles released from the mother in May 1990 were maintained in the laboratory for 1 year and used for measurements of growth (Sewell, 1993a). These individuals reproduced as males in November 1990 (Sewell, 1993a). When the growth experiment finished in May 1991, the surviving sea cucumbers were kept in the laboratory until the gonads had regrown after postspawning resorption (Sewell and Chia, 1994). In July 1991, eight animals of the surviving 78 had changed sex (unpub. data). These protandric sea cucumbers were above 185 mg TW,

Table III

Leptosynapta clarki: *Scaling constants for relationships between sea cucumber size and number of eggs or pentactulae*

Regression[1]	N	F^2	r^2	ln(a)	b	$s^2\beta$	β lower	β upper
TW *vs* # eggs	80	168.94***	0.684	−12.300	3.637	0.038	3.206	4.127
DW *vs* # eggs	80	377.55***	0.829	−237.726	6.348	0.071	5.785	6.968
Length *vs* # eggs	80	168.94***	0.684	−1050.37	61.169	0.775	53.911	69.405
ln TW *vs* ln # eggs	80	161.69***	0.675	−0.010	1.416	9.5×10^{-3}	1.246	1.610
ln DW *vs* ln # eggs	80	205.89***	0.725	0.114	1.463	9.5×10^{-3}	1.300	1.646
ln Length *vs* ln # eggs	80	138.53***	0.640	−0.589	2.465	0.011	2.154	2.821
TW *vs* # pentactulae	28	0.143 NS	0.005					
DW *vs* # pentactulae	28	15.590***	0.375	44.960	1.795	0.055	1.312	2.456
Length *vs* # pentactulae	28	0.143 NS	0.005					
ln TW *vs* ln # pentactulae	28	0.184 NS	0.007					
ln DW *vs* ln # pentactulae	28	6.644**	0.204	1.069	1.835	0.063	1.289	2.611
ln Length *vs* ln # pentactulae	28	0.122 NS	0.005					

Relationships are determined from an RMA regression on raw or log-transformed data. Formula for standard error ($s^2\beta$) and confidence intervals (β lower, β upper) on the RMA slope from McArdle (1988).

[1] TW = total weight (mg), DW = drained weight (mg).
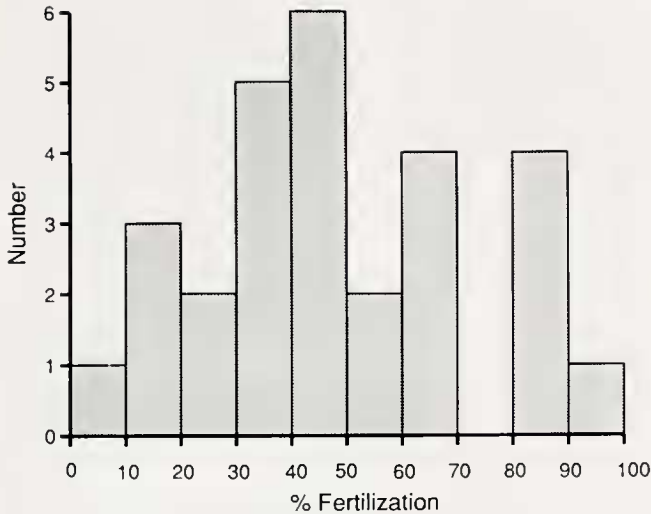
[2] NS = nonsignificant; **$p < 0.05$, ***$p < 0.001$.

Figure 5. *Leptosynapta clarki*. Number of female sea cucumbers in each percent fertilization class during November and December 1989 and 1990. $N = 28$ females.

which is about the critical size of 200–250 mg determined from field collections. The other 70 individuals, although of the same age, were male or of undetermined sex. Seven of these 70 sea cucumbers were above 185 mg TW but had not changed sex. These results suggest, therefore, that size in combination with some environmental or genetic factor may be more important than age to the timing of sex change in *L. clarki*.

Brooding and small body size

Brooding is size-dependent in many marine invertebrates, and species with smaller adults protect their embryos to more advanced stages of development (see review by Strathmann, 1990). This trend has been noted in two families of holothurians, the Cucumaridae (Menge, 1975) and the Synaptidae (Strathmann, 1990). *L. clarki* conforms to these trends in being small (adult size maximum at Bamfield is 113 mm) and brooding pentactulae in the ovary (Everingham, 1961; McEuen, 1986; Sewell and Chia, 1994).

A test of the allometry hypothesis to explain the association of small adult size with brooding (Strathmann and Strathmann, 1982) in *L. clarki* did not show proportionately smaller broods in larger sea cucumbers. The slope of the relationship between log_e drained weight and log_e number of pentactulae was not less than 1, indicating no spatial constraints on brood size in larger *L. clarki*.

Constraints on brood size can also occur if the larger broods from larger adults develop more slowly or suffer higher mortality (Strathmann and Strathmann, 1982). In *L. clarki*, despite a trend for larger females to have a higher number of pentactulae, larger females or females with a

high number of pentactulae do not show a higher degree of mortality (Sewell, 1993a). These results suggest that brood size is not constrained in larger *L. clarki* by either brood space or differential embryonic mortality.

Although the allometry hypothesis is intuitively appealing (Hess, 1993), the evidence for scaling constraints on brood size in marine invertebrates is remarkably poor. Studies that suggest that brood size is unconstrained by brood space include species of polychaetes (Daly, 1972; Gremare and Olive, 1986; Hess, 1993), bivalves (Ockelmann and Muus, 1978; Kabat, 1985; McGrath and ÓFoighil, 1986; Russell and Huelsenbeck, 1989; Brey and Hain, 1992), asteroids (Menge, 1974), ophiuroids (Rumrill, 1982; Byrne, 1991), holothuroids (Rutherford, 1973), and pinnotherid crabs (Hines, 1992). In fact, when the study on *Asterina phylactica* that provided the first empirical evidence for the hypothesis (Strathmann *et al.*, 1984) was reanalyzed with RMA regression, no scaling constraints on brooding were indicated (Hess, 1993).

One species that might show allometric constraints on brooding is the hermaphroditic, bursal-brooding *Axiognathus squamata* (Rumrill, 1982). In this species, the relationship between brood number and parental body size is significantly different from zero, but with a low slope (untransformed data $b = 0.57$; Rumrill, 1982). This indicates that smaller adults brood numbers of embryos nearly equivalent to those brooded by larger adults (Rumrill, 1982). However, in *Axiognathus squamata*, staggered oocyte maturation and brood release ensures that the production of oocytes never exceeds the capacity of the adult to brood the developing embryos (Rumrill, 1982). Therefore, the observed allometric constraint on brood size in this species may depend more upon the rate of oocyte maturation than on body size (Rumrill, 1982).

In *L. clarki*, sperm released externally enters the ovary through an as yet unknown means (Everingham, 1961; McEuen, 1987; Hess *et al.*, 1988; Sewell and Chia, 1994), but probably via the gonopore. Large amounts of sperm are available during the spawning season as a result of early reproduction, which skews the sex ratio dramatically towards males (Table II), and the production of large quantities of sperm by each male (Sewell and Chia, 1994). Although all males invest a similar proportion of energy in reproduction (about 5% of drained weight, Sewell and Chia, 1994), larger males will have larger volumes of sperm, which might result in higher fertilization success. However, even though sperm does not appear to be limiting because of high-density populations (at Bamfield, mean density = 169 per m^2; Sewell, 1993a), a skewed sex ratio, and large amounts of sperm per male, in some females fertilization is low or zero. There is also no evidence to suggest that larger females have a higher fertilization

success. If fertilization is a constraint for *L. clarki* or other brooders, then limitations on brood size due to space or embryonic requirements for dissolved materials (Strathmann and Strathmann, 1982) may never be reached.

In brooding species where fertilization success is high (*e.g., Cucumaria pseudocurata*—Rutherford, 1973; *Ophioplocus esmarki*—Rumrill, 1982; *Ophionereis olivacea*—Byrne, 1991), allometric constraints on space may be avoided by brooding of embryos to a limited stage of development (Daly, 1972; Ockelmann and Muus, 1978), three-dimensional packing of embryos in the brood space (Kabat, 1985), sequential brooding throughout the reproductive season (Kabat, 1985; Hess, 1993), or brooding within a distensible structure (Hines, 1992; present study). In the latter case, the ability for the brood structure to expand to accommodate the number of fertilized eggs might ensure that, even when fertilization success is high, all the embryos can be brooded. In such cases, a test of the allometry hypothesis may be inappropriate because, in contrast to external brooders or species with a well-defined, solid brood space, there may not be a limit to brood space.

Alternative hypotheses to explain the association between small size and brooding—such as lower energetic reserves (Chia, 1974), dispersal, or recruitment (Strathmann and Strathmann, 1982)—cannot be considered in relation to brooding in *L. clarki* until there is comparative information on other sea cucumber species. Hess (1993) has recently suggested that the slower developmental rate of embryos in large brooders may be a factor in the evolution of brooding. This time-constraint hypothesis suggests that longer developmental times may be disadvantageous because the stage-specific mortality rate is experienced over a longer period of time, and the number of broods per unit time is reduced. In the development of this hypothesis, Hess assumes that embryonic mortality may be low and relatively unimportant in brooders, but this may not be true for all brooding species (Sewell, 1993a, b). If embryonic mortality is high, as in some female *L. clarki* (Sewell, 1993a, b), then the costs of a longer development in larger brooders will be even greater (H. Hess, pers. comm.).

Although the time-constraint hypothesis may be applicable to species that spawn more than once per season, alternative hypotheses will be needed to explain the association between small size and brooding in semelparous brooders or in individuals that produce only one brood per season (Hess, 1993). As the latter category includes many echinoderms in which the association between small size and brooding is particularly pronounced (see Strathmann, 1990), this emphasizes that no single hypothesis will be applicable in all taxa (Strathmann and Strathmann, 1982).

Brooding and hermaphroditism

The association between brooding and hermaphroditism has been noted in a variety of marine invertebrates (Ghiselin, 1969, 1974; Charnov, 1982; Strathmann and Strathmann, 1982), including asteroid, ophiuroid, and holothuroid echinoderms (Ghiselin, 1969, 1974). The fairly high incidence of protandry in ophiuroids and holothuroids was believed by Ghiselin (1969) to favor the gene-dispersal model for hermaphroditism.

In the gene-dispersal model, sequential hermaphroditism can act to prevent inbreeding due to fertilization between siblings, especially in forms with restricted gene flow (Ghiselin, 1969, 1974). For example, if all members of a clutch are males at the onset of sexual maturity and change into females simultaneously, then they are never able to mate with each other (Ghiselin, 1974). The observation that there is a higher incidence of sequential hermaphroditism in forms with restricted gene flow (species with no larvae or brooders) provides some support for the hypothesis (Ghiselin, 1974), though there is little direct evidence for reduced levels of inbreeding in sequential hermaphrodites (but see Hunt, 1993), and none for brooding species. Studies on the scale of genetic differentiation in *L. clarki* (Hess *et al.,* 1988) and the confirmation of protandry in the present study may provide direct evidence for this hypothesis in a brooding species.

In an electrophoretic study of three geographically separate *L. clarki* populations on San Juan Island, Washington, Hess *et al.* (1988) found no evidence for inbreeding: the two polymorphic loci studied did not show the reduced numbers of heterozygotes expected in inbred populations. The authors concluded that there was enough movement of either individuals or gametes within each population to allow random mating and maintain heterozygosity. Similar results were obtained by Hunt (1993) in the asteroid *Patiriella exigua,* which is protandric and oviposits direct-developing eggs on the undersides of intertidal boulders (Byrne, 1992). This species has no known method for dispersal, but showed no evidence of inbreeding (Hunt, 1993). In both cases, low dispersing and protandric species, one with direct development, the other a brooder, showed evidence for random mating within isolated local populations (Hess *et al.,* 1988; Hunt, 1993).

L. clarki juveniles released from the mother rapidly form shallow burrows, and dispersal—though rare—might occur by rafting, floating, swimming, or incidental dispersal as a result of water movement (Sewell, 1993a). The combination of occasional dispersal and sequential hermaphroditism might reduce levels of inbreeding in an *L. clarki* population. However, because growth rates in juvenile *L. clarki* are extremely variable (Sewell, 1993a) and protandry appears to be dependent upon the combined effects of size and environmental/genetic effects, it

is unlikely that all siblings undergo a simultaneous sex change. Although *L. clarki* does show some evidence to support the gene-dispersal model for sequential hermaphroditism, further clarification is required to determine how far siblings disperse from one another; whether all, or only some, individuals in a clutch change sex at the same time; and if there is a reduction in inbreeding in the Bamfield population.

If we assume that sequential hermaphroditism is an advantageous strategy for *L. clarki,* the question to be answered is, Why is *L. clarki* protandric and not protogynic?

Juvenile sea cucumbers are released from the mother into the adult habitat in April or May (Sewell and Chia, in press; Sewell, 1993a). The transitional gonads with new eggs are, however, seen in sex-changing animals in January while these pentactulae were still being brooded. Therefore, if the sea cucumbers were to be reproductively active as females in their first year, they would need to start egg development while being brooded within the female. In addition, based on the regression line for drained weight *versus* number of eggs (Table III, Fig. 4), to produce any eggs at all a sea cucumber must weigh over 37 mg DW during late October. Although growth to sizes at which eggs could be produced is possible on the basis of laboratory measurements (Sewell, 1993a), the time required to produce mature eggs (10 months, Jan.–Nov.; Sewell and Chia, 1994) might be a constraint to early reproduction as a female.

In terms of the size-advantage hypothesis, although there is clearly an increase in the number of eggs produced per female with size (Fig. 4), the potential for low fertilization (as number of eggs fertilized) in all females may reduce any advantage in large females. In addition, there is a strong relationship between testis weight and male size (unpub. data), so larger males are producing larger volumes of sperm. Until the mechanism of fertilization in *L. clarki* is determined, it is not known whether male size is important to an individual's fertilization success.

Future research

Leptosynapta is an ideal genus in which to conduct further research on the association of small size, hermaphroditism, and brooding in marine invertebrates. These sea cucumbers are of small to moderate size (Hyman, 1955; 12–30 cm maximum adult length [except for *L. minuta,* 1 cm]; McEuen, 1986); are characterized by burrowing habits (Hyman, 1955); and exhibit a wide range of reproductive strategies—from lecithotrophic broadcast spawners (*L. inhaerens, L. galliennei, L. girardii, L. tenuis*) to coelomic (*L. minuta*) and ovarian brooders (*i.e.,* viviparous species: *L. clarki, L. transgressor;* for references see Smiley *et al.,* 1991). Of these species, *L. clarki* and prob-

ably *L. inhaerens* are protandric, whereas *L. minuta* is reported to be hermaphroditic (Smiley *et al.,* 1991). Further comparative work on this genus would therefore be of considerable interest because it removes phylogenetic constraints from the consideration of life history questions relating small size, brooding, and hermaphroditism.

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